nsls
IR UV X

Science Highlights

from the National Synchrotron Light Source

BEAMLINE
X4A

PUBLICATION
Priti Bachhawat, G.V.T. Swapna, Gaetano T. Montelione, and Ann M. Stock, "Mechanism of Activation for Transcription Factor PhoB Suggested by Different Modes of Dimerization in the Inactive and Active States," *Structure*, **13**, 1353-1363 (2005).

FUNDING
National Institutes of Health; NMR Core Facilities Support Award from the Cancer Institute of New Jersey

FOR MORE INFORMATION
Ann M. Stock, Department of Molecular Biology and Biochemistry, Rutgers University
stock@cabm.rutgers.edu

Mechanism of Activation for Transcription Factor PhoB Suggested by Different Modes of Dimerization in the Inactive and Active States

P. Bachhawat[1,2], G.V.T. Swapna[1,3], G.T. Montelione[1,2,3,4], and A.M. Stock[1,2]

[1]Center for Advanced Biotechnology and Medicine; [2]Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey; [3]Department of Molecular Biology and Biochemistry, Rutgers University; [4]Northeast Structural Genomics Consortium; [5]Howard Hughes Medical Institute

We have determined the crystal structures of the regulatory domain of response regulator PhoB ($PhoB_N$) in its inactive and active forms, which suggest its mechanism of phosphorylation-mediated regulation. The structure of active $PhoB_N$, together with the structure of the effector domain bound to DNA, define the conformation of the active transcription factor in which the regulatory domains dimerize using rotational symmetry while the effector domains bind to DNA tandemly, implying a lack of intra-molecular interactions. While this active DNA-bound state seems common to all members of the family, the mode of dimerization in the inactive state seems specific to PhoB.

Response regulators function within two-component systems, signal transduction pathways that are highly prevalent in bacteria. They are modular switches typically comprised of a conserved regulatory domain that regulates the activities of an associated effector domain in a phosphorylation-dependent manner. They confer virulence and antibiotic resistance in several pathogenic bacteria, making them attractive drug targets. The majority of response regulators function as transcription factors, and the OmpR/PhoB family is the largest among them.

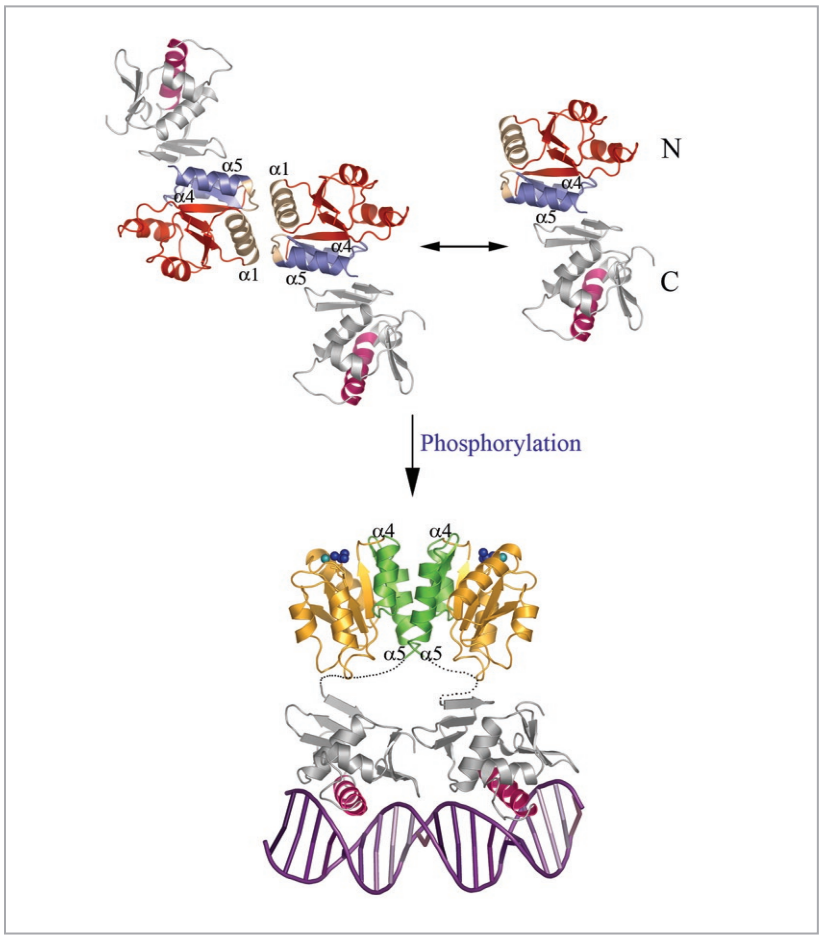
Phosphorylation at the active-site aspartate residue in the regulatory domain leads to a propagated conformational change from the active site to a distant "functional face" of the protein through the concerted reorientation of a few key residues. How this conformational change in the regulatory domain affects the activity of the effector domain in the OmpR/PhoB family is unknown. In the two published structures of full-length family members DrrB and DrrD, the recognition helix is completely exposed, unhindered by the regulatory domain, suggesting that the mechanism of activation is not intra-molecular relief of steric inhibition. We present crystallographic and solution NMR data that suggest a mechanism of activation for PhoB, and we extend it to other members of the family.

Authors (from left) Prof. Gaetano T. Montelione, Priti Bachhawat, Prof. Ann M. Stock, and Dr. GVT Swapna

The regulatory domain of PhoB shows distinct rotationally symmetric dimers in the inactive and active states when crystallized under identical conditions. In the inactive state, $PhoB_N$ crystallizes as a two-fold symmetric dimer using the α1-α5 interface. The symmetry was confirmed in solution using NMR. Concentration-dependent shifts of resonances in NMR experiments and analytical ultracentrifugation studies show that inactive $PhoB_N$ exists in equilibrium between a monomer and a dimer in solution. When the structure of the effector domain is docked on the structure of the inactive $PhoB_N$ dimer using either DrrD or DrrB as a model, the effector domains project in opposite directions, in an orientation incompatible with tandem binding to direct repeat DNA sequences. This alternate dimer is not observed for any other OmpR/PhoB family member and

may be a specific feature of PhoB that provides an additional means of regulation.

$PhoB_N$ was also crystallized in the active state using the non-covalent beryllium fluoride (BeF_3^-) complex as a phosphoryl analog. In the active state, $PhoB_N$ forms a two-fold symmetric dimer using the α4-β5-α5 interface. The symmetry was confirmed in solution using NMR. The dimer interface is composed of highly conserved residues that form a central hydrophobic patch surrounded by salt-bridges. The structure of active $PhoB_N$, together with the previously solved structure of the effector domain bound to DNA, provides a model of the active transcription factor in which the regulatory domains dimerize with rotational symmetry while the effector domains bind to DNA using tandem symmetry. The different symmetries adopted by the regulatory and effector domains suggest that activation causes a loss of intra-molecular orientational constraints on the effector domains. The high degree of conservation of the α4-β5-α5 interface and a number of other similar structures from this family suggest that this mode of dimerization is common to all members of the OmpR/PhoB family.



Model of phosphorylation-mediated activation for transcription factor PhoB